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ORIGINAL ARTICLE

Development and evaluation of a Quadruplex Taq Man real-time PCR assay for simultaneous detection of clinical isolates of *Enterococcus faecalis*, *Enterococcus faecium* and their *vanA* and *vanB* genotypes

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ABSTRACT

Background and Objectives: We developed and evaluated the utility of a quadruplex Taqman real-time PCR assay that allows simultaneous identification of vancomycin-resistant genotypes and clinically relevant enterococci.
Materials and Methods: The specificity of the assay was tested using reference strains of vancomycin-resistant and susceptible enterococci. In total, 193 clinical isolates were identified and subsequently genotyped using a Quadruplex Taqman real-time PCR assay and melting curve analysis. Representative Quadruplex Taqman real-time PCR amplification curve were obtained for *Enterococcus faecium*, *Enterococcus faecalis*, vanA-containing *E. faecium*, vanB-containing *E. faecalis*.
Results: Phenotypic and genotypic analysis of the isolates gave same results for 82 enterococcal isolates, while in 5 isolates, they were inconsistent. We had three mixed strains, which were detected by the TaqMan real-time PCR assay and could not be identified correctly using phenotypic methods.
Conclusion: Vancomycin resistant enterococci (VRE) genotyping and identification of clinically relevant enterococci were rapidly and correctly performed using TaqMan real-time multiplex real-time PCR assay.

Key words: Enterococci, Vancomycin, Multiplex TaqMan real-time PCR

INTRODUCTION

Enterococci are one of the major causes of hospital-acquired infections although they can also cause human infections in the community (1). Hospital acquired infection is defined as an infection which develops 48 h after hospital admission not being the reason of the admission. Enterococci are individual, paired, or short-chain Gram-positive, catalase-negative cocci. This organism is mainly commensals in gastrointestinal tract of healthy individuals but may become opportunistic pathogens in immune-compromised hosts and in patients who have received broad-spectrum antimicrobial therapy or had a prolonged hospital stay (2).

Enterococci display both intrinsic and acquired resistance patterns to many antimicrobials, such as glycopeptides, β-lactams, fluoroquinolones and aminoglycosides which dramatically reduce the remaining therapeutic options among patients infected with these organisms (3). Along with *E. faecalis*, the genus *Enterococcus* includes *E. faecium* which found less frequently than *Enterococcus faecalis* in clinical isolates and are significantly more resistant to vancomycin than *E. faecalis*.

E. faecalis and *E. faecium* are the main causative agents for serious relevant nosocomial infection in humans, thereby it is necessary to discriminate between the low-level vancomycin resistant *E. faecalis* and *E. faecium* isolates with the other low-virulence motile enterococcal species (4, 5). Moreover, since vancomycin offers as last line of

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